

Remarks

The pending claims in the application are 13, 14, 18-20, 29, 52, 53, and 58-68.

The Claims

Claims 29 and 60-62 properly recite “transgenic mammal” or “mammal.” The clean version of the pending claims attached to the response filed September 26, 2002 mistakenly recited “transgenic animal” or “animal.” The claims should have recited “transgenic mammal” or “mammal” because an amendment filed January 7, 2002 entered this recitation in claims 29 and 60-62 and it was not subsequently amended.

Claims 63-65 have been added and recite that the nonhuman, transgenic, mammal of claims 60-62 is a mouse. New claims 66-68 recite that the nonhuman, transgenic, mammal of claims 60-62 is a cow. These claims are supported by the specification which discloses that “transgenic animals can be prepared from domestic livestock, *e.g.*, cows, pigs, sheep, goats, horses, etc. . . . or experimental animals for research or product testing, *e.g.*, mice, rats, hamsters, guinea pigs, rabbits, etc.” (Page 9, lines 16-20.)

The amendments introduce no new matter

Background to the invention

The inventors discovered a human dominant negative allele of PMS2 that was associated with hereditary non-polyposis colon cancer (HNPCC). A dominant negative form of a gene interferes with the function of a wild type gene. (See Lodish *et al.*, Molecular Cell Biology (2000) page 3; Exhibit A.) Applicants demonstrated that this dominant negative form of the PMS2 gene could be transfected into mammalian cells. The cells transfected with the dominant negative form of the PMS2 gene became

“hypermutable” mammalian cells. The specification discloses, “Dominant negative alleles of such genes [mismatch repair genes], when introduced into cells or transgenic animals, increase the rate of spontaneous mutations by reducing the effectiveness of DNA repair and thereby render the cells or animals hypermutable.” (Page 6, lines 16-19.) The transfected cells had a disrupted mismatch repair system which caused an increase in the rate of mutation.

The rejections under 35 U.S.C. §112, first paragraph

The Office Action rejects the claims under 35 U.S.C. §112, first paragraph, as not enabled by the specification and for lack of adequate written description. The Office Action states that the arguments previously submitted are not persuasive, and states that the Applicants did not address the concerns raised in the previous arguments. Applicants respectfully traverse.

Enablement

The Office Action asserts that the claimed invention is not enabled because applicants have not proven that any transgenic animal can be made using the teachings in the specification. Applicants provided the declaration of Dr. Kline to demonstrate that the teachings of the specifications could be used to produce a transgenic mammal, *e.g.*, a transgenic mouse. The Office Action has asserted, however, that the declaration of Dr. Kline “only indicates that a transgenic mouse was made and therefore, there is no evidence that any transgenic animal (non-human) could be made.” (Paper 15, page 3, lines 12-13.)

Methods of making transgenic mice and cattle were known prior to the effective filing date of the application, April 14, 1998. Exhibits B-F teach methods of making

these transgenic mammals that were known before the effective filing date of the application.

Bremel *et al.* (U.S. Patent 6,080,912, filed March 20, 1997; Exhibit B) teaches at Examples 5 and 7 how to produce transgenic cattle. At Example 5, Bremel teaches isolation, preparation, and microinjection of bovine oocytes and zygotes with a transgene of interest. See column 18, line 25 to column 20, line 16. At Example 7, Bremel further teaches preparation of surrogate cows for introduction of the microinjected oocytes and zygotes. See column 21, lines 7-20. Bremel then teaches the transfer of the microinjected oocytes and zygotes into the surrogate cows. See column 21, lines 29-32. Using the detailed procedure set forth in Examples 5 and 7 Bremel found that transgenic calves were produced from infected pre-fertilization oocytes.

In the first transfer 8 embryos derived from infected pre-fertilization oocyte were transferred into 4 recipients; four calves were born to these recipients and all four were found to be positive for the presence of vector proviral DNA (i.e., 100% were transgenic). In the second transfer 8 embryos derived from pre-fertilization oocytes were transferred into 4 recipients; 2 calves were born to these recipients and one of these animals was found to be transgenic (in the second transfer, one pregnancy was lost in the first month and another pregnancy comprising twins was lost in the eighth month; neither embryo from the 8 month pregnancy was transgenic).

Column 21, lines 36-47. Thus Bremel teaches a successful method for production of transgenic cattle.

Deboer *et al.* (U.S. Patent 6,140,552, filed June 7, 1995; Exhibit C) also teaches a method for producing transgenic cattle. Deboer teaches that the method is performed by collecting bovine oocytes (column 40, lines 26-40), fertilizing the oocytes (column 40, line 47 to column 41, line 28), microinjecting DNA containing the transgene into the

fertilized oocytes (column 41, lines 51-64), culturing of the microinjected fertilized oocytes (column 41, line 65 to column 42, line 17), and transferring the cultured oocytes into a surrogate cow (column 42, lines 18-51). Deboer teaches that transgenic calves are generated using this detailed protocol. "Calf #15 (a female) was mosaic for integration of the transgene. Placental tissue was positive, whereas in blood and ear tissue no hLF sequences could be detected. . . . Calf #4 (a male) showed, in all three tissues, the same hybridization pattern that was identical to the expected one." (Column 43, lines 47-62.)

Sun (U.S. Patent 6,339,183, filed November 13, 1997; Exhibit D) teaches production of transgenic mice containing the fusion gene uroplakin II-lacZ (UPII-lacZ). At Example 2 Sun teaches, "A 6-kb XhoI fragment of the G1 genomic clone (FIG. 1a) was subcloned in pGEM7Z and then restriction-cut to yield a 3.6-kb DNA fragment of G1 clone (extending from the XhoI site at -3.6 kb to the BamHI site at -42 bp relative to the transcription initiation site) and inserted into the SmaI site of a lacZ vector, placF to generate pUPII-LacZ (FIG. 3). The 7.1-kb fusion gene was excised using Kpn I and Hind III, gel-purified, and microinjected into fertilized mouse eggs (from F1 hybrids of C57BL/6JxDBA2), which were implanted into CD-1 foster mothers. The lacZ transgene was identified by Southern blot analysis of tail DNA in accordance with methods well known in the art. Positive founder animals were back-crossed with (C57BL/6JxDBA2) F1 hybrids to generate hemizygous animals that were used for studying transgene expression." (Column 9, line 9 to column 10, line 6.) Thus Sun teaches how to produce transgenic mice.

Bujard (U.S. Patent 5,912,411, filed June 7, 1995; Exhibit E) also teaches production of transgenic mice. Bujard teaches:

Mice expressing tTA protein were obtained by pronuclear injection into fertilized oocytes of a 2.7 kb XhoI-PfmI fragment excised from plasmid pUHG17-1. This DNA fragment contained the tTA^R gene (shown in SEQ ID NO: 1) under the transcriptional control of the human CMV 1E promoter (position +75 to -675) together with a rabbit β -globin polyadenylation site including an intron. The human CMV 1E promoter is a constitutive promoter that allows expression of the mutated tetR-VP16 fusion protein in all cells where chromosomal integration of the DNA sequence encoding tTA^R has occurred. DNA was injected into fertilized oocytes at a concentration of approximately 5 ng per μ l by standard techniques. Transgenic mice were generated from the injected fertilized oocytes according to standard procedures. Transgenic founder mice were analyzed using polymerase chain reaction (PCR) and Southern hybridization to detect the presence of the tTA^R transgene in chromosomal DNA of the mice. Two transgenic mouse lines, CR3 and CR4 were identified and crossbred with another transgenic mouse line carrying a luciferase reporter gene under the control of tetO sequences.

Column 52, lines 35-55.

Houdebine *et al.* (U.S. Patent 5,965,788, PCT filed June 12, 1992; Exhibit F)

teaches generation of transgenic mice. Houdebine teaches:

The pW₃ [encoding the promoter of the rabbit WAP gene and the human growth hormone] and pJ₄ [encoding the rabbit WAP gene and the bovine growth hormone gene] fragments were used to obtain transgenic animals. Transgenic mice were obtained by the conventional technique of microinjection (Brinster *et al.*, Proc. Natl. Acad. Sci. U.S.A. (1985) 82, 4438-4442). 1-2-pl containing 500 copies of the gene were injected into the male pronucleus of mouse embryos. The constructs were prepared in the vector p-polyIII-I (Lathe *et al.*, Gene (1987) 57, 193-201). The NotI-NotI fragments of this vector containing the recombinant genes were microinjected. The embryos were then transferred into the oviduct of hormonally prepared adoptive females. About 10% of the engineered embryos gave birth to young mice and 2-5% of the engineered embryos to transgenic young mice. The presence of the transgenes was revealed by the technique of

Southern blotting form the DNA extracted from the mouse tails.

Column 7, lines 13-26.

Further, the Declaration of Dr. Kline provides factual evidence that one of skill in the art could make transgenic animals using the teaching of the specification. Thus the Specification, as discussed above describes how to make the claimed transgenic mammals.

The Patent Office has cited several research articles in support of its position that the claims are not enabled for all mammals. However, these references do not demonstrate that one of skill in the art would have to resort to undue experimentation to make the claimed mammals. As stated in Applicants' response filed January 7, 2002, the references merely point out possible inconveniences in producing transgenic animals in particular species.

The Patent Office asserts that even if the specification teaches one of skill in the art how to make the claimed transgenic mammals, it does not teach the skilled artisan how to use the transgenic mammals. The specification discloses that the claimed mammals can be used to screen for mutations in genes of interest. The mammals can be used as the basis of animal models for investigating the function of a protein *in situ*. The specification discloses,

Once a transfected cell line or a colony of transgenic animals has been produced, it can be used to generate new mutations in one or more gene(s) of interest. A gene of interest can be any gene naturally possessed by the cell line or transgenic animal or introduced into the cell line or transgenic animal. An advantage of using such cells or animals to induce mutations is that the cell or animal need not be exposed to mutagenic chemicals or radiation, which

may have secondary harmful effects, both on the object of the exposure and on the workers.

Page 10, lines 7-14. Thus, a hypermutable mammal accumulates mutations at a greater frequency than control animals. These mutations occur in genes of interest at any position in the hypermutable mammal's genome or in a DNA construct introduced into the cells of the mammal. An investigator identifies these genes, and the effects of these mutations on the mammal can be studied. The introduction of mutations in the claimed mammal is advantageous over prior art methods which used chemicals or radiation. These mammals introduce mutations without harsh chemicals and radiation, limiting the exposure of laboratory personnel to such mutagens.

The Office Action also asserts that it is not clear how the hypermutable animals would be used to study the effects of a mutation in a gene of interest. "[T]he specification does not provide any guidance as to how the animals would be used for the intended utility. For example, if a DNA was to be introduced in an animal, how would it be administered to the animal and what dose would be used and how would the mutation be monitored." (Paper 10, page 6, lines 3-6.) The specification teaches how to make and use the claimed hypermutable transgenic mammal to study a mutation in a gene of interest. The specification teaches how to administer DNA containing the dominant negative allele to a mammal. "The polynucleotide can be introduced into the cell by transfection." (Page 8, lines 11-12.) The specification discloses how to transfect cells. The specification discloses, "Techniques for transfection are well known. Available techniques for introducing polynucleotides include but are not limited to electroporation, transduction, cell fusion, the use of calcium chloride, and packaging of the polynucleotide together with lipid for fusion with the cells of interest." (Page 8, lines 25-

29.) Details of these methods were well-known in the art at the time the application was filed.

The specification also discloses how to monitor mutations in genes of interest.

The specification discloses,

Mutations can be detected by analyzing for alterations in the genotype of the cells or animals, for example by examining the sequence of genomic DNA, cDNA, messenger RNA, or amino acids associated with the gene of interest. Mutations can also be detected by screening the phenotype of the gene. A mutant phenotype can be detected by identifying alterations in electrophoretic mobility, spectroscopic properties, or other physical or structural characteristics of a protein encoded by a mutant gene. One can also screen for alteration of any property of the cell or animal associated with the function of the gene of interest.

Page 10, lines 15-24. It is standard and routine in the field of molecular biology to analyze a gene of interest to detect a mutation.

The Office Action further asserts that one of skill in the art would have been unable to use the claimed mice because the phenotype of the mice is not disclosed in the specification. The Office Action inquired: "When the dominant negative form of the PMS2 is expressed in animals, what would be the effect on the metabolism of these animals or what would be the phenotype of these animals." (Paper 10, page 5, lines 15-17.) A phenotype is defined as: "The observable characteristics, at the physical, morphologic, or **biochemical** level, of an individual, as determined by the genotype and environment." (Stedman's Online Medical Dictionary, 27th Edition, emphasis added; Exhibit G.) Thus, the phenotype of the transgenic mice is described by a biochemical difference from wild type mice. The instant application describes a biochemical difference in the mammals that are transgenic for a dominant negative mismatch repair

gene. The biochemical difference is an increased mutation rate and accumulation of mutations relative to wild type mammals.

The specification discloses this phenotype: “A cell or an **animal** into which a dominant negative allele of a mismatch repair gene has been introduced will become hypermutable. This means that the spontaneous mutation rate of such cells or **animals** is elevated compared to cells or animals without such alleles.” (Page 7, lines 28-31, emphasis added.) Thus, these animals have a phenotype that is disclosed in the application. The phenotype is also recited in the claims. The independent claims each recite, “A **hypermutable**, nonhuman transgenic mammal” or “A method of making a **hypermutable**, nonhuman, mammalian, fertilized egg.”

Furthermore, a supplemental declaration of Dr. Kline accompanies this response. (Exhibit H.) The supplemental declaration provides further evidence that the claimed mammals have the phenotype described in the specification and recited in the claims. Specifically, the animals exhibit microsatellite instability, which is a hallmark of hypermutability.

The specification teaches one of skill in the art how to make and use the claimed hypermutable transgenic mammals. Withdrawal of the enablement rejection to claims 13, 14, 18-20, 29, 52, 53, and 58-62 is respectfully requested.

Written Description:

The Office Action alleges that the specification inadequately describes the claimed subject matter. The Written Description Requirement may be fulfilled by a description of “sufficiently detailed, relevant identifying characteristics.” “[C]omplete or partial structure, other physical and/or chemical properties, functional characteristics

when coupled with a known or disclosed correlation between function and structure, or some combination of such characteristics” may be used to satisfy the Written Description Requirement (Fed. Register, Vol. 66(4):1106, Col. 1, Jan. 5, 2001).

As discussed above, the specification describes the claimed hypermutable animals including their phenotype. The specification discloses that the transgenic animals comprise a dominant negative allele of a mismatch repair gene. The specification discloses, “A polynucleotide encoding a dominant negative form of a mismatch repair protein can be introduced into the genome of an animal by producing a transgenic animal.” (Page 9, lines 12-14.) The specification also describes such a dominant negative form of the mismatch repair gene. The specification discloses, “An example of a dominant negative allele of a mismatch repair gene is the human gene *hPMS2-134*, which carries a truncation mutation at codon 134.” (Page 7, lines 2-4.) Moreover, the Specification provides “sufficiently detailed, relevant identifying characteristics” of the transgenic animals. The specification discloses:

A cell or animal into which a dominant negative allele of a mismatch repair gene has been introduced will become hypermutable. This means that the spontaneous mutation rate of such cells or animals is elevated compared to cells or animals without such alleles.

Page 7, lines 28-31.

The specification also provides “[C]omplete or partial structure, other physical and/or chemical properties, functional characteristics ... coupled with a known or disclosed correlation between function and structure....” That is, the Specification teaches the structure of a dominant negative allele of a mismatch repair gene, *PMS2-134*, and its function in mammalian cells as a dominant negative allele. The specification

provides working examples demonstrating that the dominant negative allele, when introduced into mammalian cells exerts a dominant negative phenotype. See Example 1, which describes how the dominant negative allele of *PMS2*, originally discovered in human patients with HNPCC, was introduced into Syrian hamster cells ("SH cells") and assayed for mismatch repair activity. The data in the working example 1 demonstrates "a similar dominant negative effect of the hPMS2-134 protein in both rodent and human systems and validates the utility of the rodent system in these studies." (Page 13, lines 28-30.) Further, working example 2 demonstrates that the *PMS2* dominant negative allele causes a defect in mismatch repair activity in rodent cells. (Page 17, lines 15-17.)

Thus the Applicants working examples show that a dominant negative allele of one mammal works similarly in a different mammal. The specification teaches the structure of an exemplary form of a dominant negative mismatch repair gene and provides biochemical characteristics and functional data regarding the hypermutable phenotype of cells and animals carrying the dominant negative form of the mismatch repair gene. The correlation of structure and function is shown by the demonstration that a human dominant negative mismatch repair protein leads to impaired mismatch repair activity in humans and, when the same gene is transferred to rodents, it causes the same impairment.

Thus, the Applicants have provided an adequate written description of the claimed invention such that one of skill in the art would recognize that they were in possession of the invention at the time of filing. Withdrawal of this rejection to claims 13, 14, 18-20, 29, 52, 53, and 58-62 is respectfully requested.

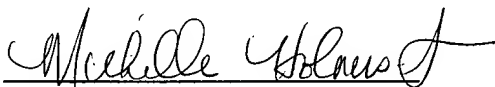
Applicants earnestly submit that the claims are enabled by the specification and are supported by an adequate written description based on the specification, the factual evidence provided in the Declaration of Dr. J. Bradford Kline, and the comments presented previously and herein. Applicants respectfully request prompt allowance of the claims.

Double Patenting

The Applicants note the provisional double patenting rejection, and will address the rejection accordingly upon indication of allowable claims. Abeyance of this rejection is respectfully requested.

Respectfully submitted,

Dated: April 18, 2003

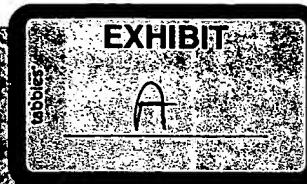
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MOLECULAR CELL BIOLOGY

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Fourth Edition W. H. FREEMAN,
41 Madison Avenue, New York, New York 10010 and Houndsmills,
Basingstoke RG21 6XS, England ISBN 0-7167-3136-3 1986. 1990. 1995.
2000. by W. H. Freeman and Company Molecular cell biology / Harvey
Lodish p [et al.] – 4th ed. p. cm. Includes bibliographical references. ISBN
0-7167-3136-3 1. Cytology. 2. Molecular biology. I. Lodish, Harvey
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MOLECULAR CELL BIOLOGY

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8.1. Mutations: Types and Causes

The development and function of an organism is in large part controlled by genes. Mutations can lead to changes in the structure of an encoded protein or to a decrease or complete loss in its expression. Because a change in the DNA sequence affects all copies of the encoded protein, mutations can be particularly damaging to a cell or organism. In contrast, any alterations in the sequences of RNA or protein molecules that occur during their synthesis are less serious because many copies of each RNA and protein are synthesized.

Geneticists often distinguish between the genotype and phenotype of an organism. Strictly speaking, the entire set of genes carried by an individual is its genotype, whereas the function and physical appearance of an individual is referred to as its phenotype. However, the two terms commonly are used in a more restricted sense: genotype usually denotes whether an individual carries mutations in a single gene (or a small number of genes), and phenotype denotes the physical and functional consequences of that genotype.

Mutations Are Recessive or Dominant

A fundamental genetic difference between organisms is whether their cells carry a single set of chromosomes or two copies of each chromosome. The former are referred to as **haploid**; the latter, as diploid. Many simple unicellular organisms are haploid, whereas complex multicellular organisms (e.g., fruit flies, mice, humans) are diploid.

Different forms of a gene (e.g., normal and mutant) are referred to as alleles. Since diploid organisms carry two copies of each gene, they may carry identical alleles, that is, be homozygous for a gene, or carry different alleles, that is, be heterozygous for a gene. A recessive mutation is one in which both alleles must be mutant in order for the mutant phenotype to be observed; that is, the individual must be homozygous for the mutant allele to show the mutant phenotype. In contrast, the phenotypic consequences of a dominant mutation are observed in a heterozygous individual carrying one mutant and one normal allele (Figure 8-1).

Recessive mutations inactivate the affected gene and lead to a *loss of function*. For instance, recessive mutations may remove part of or all the

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gene from the chromosome, disrupt expression of the gene, or alter the structure of the encoded protein, thereby altering its function. Conversely, dominant mutations often lead to a *gain of function*. For example, dominant mutations may increase the activity of a given gene product, confer a new activity on the gene product, or lead to its inappropriate spatial and temporal expression. Dominant mutations, however, may be associated with a loss of function. In some cases, two copies of a gene are required for normal function, so that removing a single copy leads to mutant phenotype. Such genes are referred to as *haplo-insufficient*. In other cases, mutations in one allele may lead to a structural change in the protein that interferes with the function of the wild-type protein encoded by the other allele. These are referred to as *dominant negative mutations*.


Some alleles can be associated with both a recessive and a dominant phenotype. For instance, fruit flies heterozygous for the mutant *Stubble* (*Sb*) allele have short and stubby body hairs rather than the normal long, slender hairs; the mutant allele is dominant in this case. In contrast, flies homozygous for this allele die during development. Thus the recessive phenotype associated with this allele is lethal, whereas the dominant phenotype is not. [↑ TOP](#)


Inheritance Patterns of Recessive and Dominant Mutations Differ


Recessive and dominant mutations can be distinguished because they exhibit different patterns of inheritance. To understand why, we need to review the type of cell division that gives rise to gametes (sperm and egg cells in higher plants and animals). The body (somatic) cells of most multicellular organisms divide by mitosis (see [Figure 1-10](#)), whereas the germ cells that give rise to gametes undergo meiosis. Like body cells, premeiotic germ cells are diploid, containing two of each morphologic type of chromosome. Because the two members of each such pair of homologous chromosomes are descended from different parents, their genes are similar but not usually identical. Single-celled organisms (e.g., the yeast *S. cerevisiae*) that are diploid at some phase of their life cycle also undergo meiosis (see [Figure 10-54](#)).


[Figure 8-2](#) depicts the major events in meiosis. *One* round of DNA replication, which makes the cell $4n$, is followed by *two* separate cell divisions, yielding four haploid ($1n$) cells that contain only one chromosome of each homologous pair. The apportionment, or segregation, of homologous chromosomes to daughter cells during the first meiotic division is random; that is, the maternally and paternally derived members of each pair, called homologs, segregate independently, yielding germ cells with different mixes of paternal and maternal chromosomes. Thus parental characteristics are reassorted randomly into each new germ cell during meiosis. The number of possible varieties of meiotic segregants is 2^n , where n is the haploid number of chromosomes. In the case of a single chromosome, as illustrated in [Figure 8-2](#), meiosis gives rise to two types of gametes; one type carries the maternal homolog and the other carries the paternal homolog.

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Now, let's see what phenotypes are generated by mating of wild-type individuals with mutants carrying either a dominant or a recessive mutation. As shown in [Figure 8-3a](#), half the gametes from an individual heterozygous for a dominant mutation in a particular gene will have the wild-type allele, and half will have the mutant allele. Since fertilization of female gametes by male gametes occurs randomly, half the first filial (F_1) progeny resulting from the cross between a normal wild-type individual and a mutant individual carrying a single dominant allele will exhibit the mutant phenotype. In contrast, all the gametes produced by a mutant homozygous for a recessive mutation will carry the mutant allele. Thus, in a cross between a normal individual and one who is homozygous for a recessive mutation, none of the F_1 progeny will exhibit the mutant phenotype ([Figure 8-3b](#)). However, one-fourth of the progeny from parents both heterozygous for a recessive mutation will show the mutant phenotype. [↑ top](#)

Mutations Involve Large or Small DNA Alterations

A mutation involving a change in a single base pair, often called a point mutation, or a deletion of a few base pairs generally affects the function of a single gene ([Figure 8-4a](#)). Changes in a single base pair may produce one of three types of mutation:

- • *Missense mutation*, which results in a protein in which one amino acid is substituted for another
- • *Nonsense mutation*, in which a stop codon replaces an amino acid codon, leading to premature termination of translation
- • *Frameshift mutation*, which causes a change in the reading frame, leading to introduction of unrelated amino acids into the protein, generally followed by a stop codon

Small deletions have effects similar to those of frameshift mutations, although one third of these will be in-frame and result in removal of a small number of contiguous amino acids.

The second major type of mutation involves large-scale changes in chromosome structure and can affect the functioning of numerous genes, resulting in major phenotypic consequences. Such *chromosomal mutations* (or abnormalities) can involve deletion or insertion of several contiguous genes, inversion of genes on a chromosome, or the exchange of large segments of DNA between nonhomologous chromosomes ([Figure 8-4b](#)). [↑ top](#)

Mutations Occur Spontaneously and Can Be Induced

Mutations arise spontaneously at low frequency owing to the chemical instability of purine and pyrimidine bases and to errors during DNA replication. Natural exposure of an organism to certain environmental factors, such as ultraviolet light and chemical carcinogens (e.g., aflatoxin

B1), also can cause mutations.

A common cause of spontaneous point mutations is the deamination of cytosine to uracil in the DNA double helix. Subsequent replication leads to a mutant daughter cell in which a T·A base pair replaces the wild-type C·G base pair. Another cause of spontaneous mutations is copying errors during DNA replication. Although replication generally is carried out with high fidelity, errors occasionally occur. [Figure 8-5](#) illustrates how one type of copying error can produce a mutation. In the example shown, the mutant DNA contains nine additional base pairs.

In order to increase the frequency of mutation in experimental organisms, researchers often treat them with high doses of chemical mutagens or expose them to ionizing radiation. Mutations arising in response to such treatments are referred to as *induced* mutations. Generally, chemical mutagens induce point mutations, whereas ionizing radiation gives rise to large chromosomal abnormalities.

Ethylmethane sulfonate (EMS), a commonly used mutagen, alkylates guanine in DNA, forming *O*⁶-ethylguanine ([Figure 8-6a](#)). During subsequent DNA replication, *O*⁶-ethylguanine directs incorporation of deoxythymidylate, not deoxycytidylate, resulting in formation of mutant cells in which a G·C base pair is replaced with an A·T base pair ([Figure 8-6b](#)). The causes of mutations and the mechanisms cells have for repairing alterations in DNA are discussed further in [Chapter 12](#). [↑ TOP](#)

Some Human Diseases Are Caused by Spontaneous Mutations



Many common human diseases, often devastating in their effects, are due to mutations in single genes. Genetic diseases arise by spontaneous mutations in germ cells (egg and sperm), which are transmitted to future generations. For example, *sickle-cell anemia*, which affects 1 in 500 individuals of African descent, is caused by a single missense mutation at codon 6 of the β -globin gene; as a result of this mutation, the glutamic acid at position 6 in the normal protein is changed to a valine in the mutant protein. This alteration has a profound effect on hemoglobin, the oxygen-carrier protein of erythrocytes, which consists of two α -globin and two β -globin subunits (see [Figure 3-11](#)). The deoxygenated form of the mutant protein is insoluble in erythrocytes and forms crystalline arrays. The erythrocytes of affected individuals become rigid and their transit through capillaries is blocked, causing severe pain and tissue damage. Because the erythrocytes of heterozygous individuals are resistant to the parasite causing malaria, which is endemic in Africa, the mutant allele has been maintained. It is not that individuals of African descent are more likely than others to acquire a mutation causing the sickle-cell defect, but rather the mutation has been maintained in this population by interbreeding.

Spontaneous mutation in somatic cells (i.e., non-germline body cells) also is an important mechanism in certain human diseases, including

retinoblastoma, which is associated with retinal tumors in children (see [Figure 24-11](#)). The hereditary form of retinoblastoma, for example, results from a germ-line mutation in one *Rb* allele and a second somatically occurring mutation in the other *Rb* allele ([Figure 8-7a](#)). When an *Rb* heterozygous retinal cell undergoes somatic mutation, it is left with no normal allele; as a result, the cell proliferates in an uncontrolled manner, giving rise to a retinal tumor. A second form of this disease, called *sporadic retinoblastoma*, results from two independent mutations disrupting both *Rb* alleles ([Figure 8-7b](#)). Since only one somatic mutation is required for tumor development in children with hereditary retinoblastoma, it occurs at a much higher frequency than the sporadic form, which requires acquisition of two independently occurring somatic mutations. The *Rb* protein has been shown to play a critical role in controlling cell division ([Chapter 13](#)).

In a later section, we will see how normal copies of disease-related genes can be isolated and cloned. [↑ TOP](#)

SUMMARY

- Diploid organisms carry two copies (alleles) of each gene, whereas haploid organisms carry only one copy.
- Mutations are alterations in DNA sequences that result in changes in the structure of a gene. Both small and large DNA alterations can occur spontaneously. Treatment with ionizing radiation or various chemical agents increases the frequency of mutations.
- Recessive mutations lead to a loss of function, which is masked if a normal copy of the gene is present. For the mutant phenotype to occur, both alleles must carry the mutation.
- Dominant mutations lead to a mutant phenotype in the presence of a normal copy of the gene. The phenotypes associated with dominant mutations may represent either a loss or a gain of function.
- In meiosis, a diploid cell undergoes one DNA replication and two cell divisions, yielding four haploid cells ([Figure 8-2](#)). The members of each pair of homologous chromosomes segregate independently during meiosis, leading to the random reassortment of maternal and paternal alleles in the gametes.
- Dominant and recessive mutations exhibit characteristic segregation patterns in genetic crosses (see [Figure 8-3](#)).

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phenotype (fē' nō-tīp)

The observable characteristics, at the physical, morphologic, or biochemical level, of an individual, as determined by the genotype and environment.

[G. *phainō*, to display, + *typos*, model]

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